

OPTIMIZATION OF BIODIESEL PRODUCTION FROM JATROPHA OIL
USING IMMOBILIZED LIPASE

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To my beloved family

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ABSTRACT

The objective of this study was to produce biodiesel through an enzymatic transesterification process. In this process, jatropha oil was used as the feedstock to react with methanol in the presence of *Rhizopus oryzae* lipase catalyst. *Rhizopus oryzae* lipase was immobilized with an innovated method to avoid inhibition and deactivation effects of glycerol and alcohol. The Lipase immobilization, in turn, made the process of separation much easier in comparison with utilizing free lipase. The effect of four important variables (time, temperature, alcohol/oil molar ratio, and oil moisture content) on the yield of biodiesel was studied. The biodiesel production process was modeled and optimized using Artificial Neural Network (ANN), Response Surface Methodology (RSM), and Genetic Algorithm (GA). Maximum yield percentage of 87.07 and 86.62% was predicted by ANN and RSM, respectively. The experimental amount of 87.1% was achieved at optimum parameters: reaction time of 17 h, temperature of 40 °C, 70 % (wt %) water content and the alcohol/oil molar ratio of 5 %. The physico-chemical properties of the jatropha oil and obtained biodiesel were investigated and compared to ASTM D6751 standard. It was observed that the quality of the produced biodiesel is in an acceptable range. The results of the present study show that the immobilization method is reliable and can enhance the performance of the lipase as the catalyst for biodiesel production with moderate conditions.

ABSTRAK

Dalam kajian ini, kaedah yang digunakan ialah kaedah transesterifikasi dan bahan mentah yang digunakan ialah minyak jatropha. Minyak jatropha bukan minyak masak dan ini dapat mengurangkan masalah yang timbul dalam perindustrian pembuatan biodisel. Biomangkin digunakan untuk mengurangkan masalah yang timbul akibat penggunaan mangkin asid dan alkali, serta membuatkan proses ini lebih mesra alam. Lipase rhizopus oryzae telah digunakan sebagai mangkin; lipase ini dinyahaktifkan untuk membuatnya lebih efisien dan membolehkan proses pengasingan mangkin menjadi lebih mudah. Kualiti hasil turut meningkat disebabkan penyahaktifan ini. Dalam kaedah transesterifikasi, alcohol digunakan untuk proses pengalkoholan dan methanol telah digunakan. Empat pembolehubah telah dipilih untuk kajian ini, iaitu masa, suhu, nisbah mol alcohol kepada minyak dan kandungan air. Proses penghasilan biodisel sangat memakan masa dan memerlukan kos yang tinggi. Oleh itu, pencarian keadaan yang optimum untuk proses ini dijalankan amat penting untuk mengurangkan kos serta masa. Metodologi permukaan bertindakbalas serta rangkaian neural tiruan digunakan untuk membuat simulasi proses pembuatan biodisel. Algoritma genetik turut digunakan untuk mengenalpasti keadaan yang optimum. Dalam kajian ini, hasil proses yang maksimum dapat dicapai sebanyak 87.10% pada keadaan optimum 40°C, 17 jam, 5% nisbah metanol/minyak dan 70 (wt %) kandungan air. Keputusan daripada ANN dan RSM masing masing adalah 86.62% dan 87.07%.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ANN	Artificial Neural Network
ASTM	American Society of Testing and Materials
BDF	Biodiesel Fuel
CCD	Central Composite Design
DG	Diglycerides
E	Ester
FAME	Fatty Acid Methyl Ester
FESEM	Field Emission Scanning Electron Microscopy
FFA	Free Fatty Acid
GL	Glycerol
GC	Gas Chromatograph
GC-MS	Gas Chromatograph-Mass Spectroscopy
ME	Methyl Ester
MeOH	Methanol
MG	Mono Glyceride
ROL	Rhizopus Oryzae Lipase
RSM	Response Surface Methodology
TG	Triglyceride
WCO	Waste Cooking Oil

CHAPTRE 1

INTRODUCTION

1.1 Background of research

There are variety of reasons which are encouraged the researchers to find the renewable and alternative sources of energy. As the most important reasons, it can be mentioned to environmental concerns, fossil fuel depletion, increasing the price of petroleum and rising energy demand (Demirbas, 2009).

Fatty acid methyl ester (FAME), known as biodiesel, has attracted vast attention in recent years and is potentially one of the main sources of fuel in the near future. Biofuel and biomass-based energy have potential to become major contributors of energy in the next century. Currently about 90% of the biofuel market is captured by bioethanol and biodiesel. Biodiesel is a fuel derived from renewable resources, such as vegetable oils (Sharma *et al.* 2008; Ma and Hanna 1999). However, the industrial scale production of biodiesel is limited due to the unwanted byproducts, restoration of glycerol, withdrawal of inorganic water and salts, wastewater treatment and high energy requirement.

Biodiesel production developed by Rudolph Diesel in the 1890s. He worked on vegetable oil fuels and shows that pure vegetable oils could be used as the fuel for early diesel engines in agriculture and some areas of world where at that time they did not have access to the petroleum. The kind of modern fuel that is known as biodiesel is produced by conversion of oils such as vegetable oils into FAMES.

The focus of industrial production is on reconstructing vegetable oils into a mixture of fatty acid esters by a process commonly described as being akin to the cracking of petroleum which is triglycerides transesterification with low molecular weight alcohols. This reaction is triglyceric esters alcoholysis which produces a mixture with higher volatility (biodiesel) that its physical properties are much more alike to those of conventional diesel fluids. The sequence of transesterification process is illustrated in Figure 1.1 (Srivathsan *et al.*, 2008).

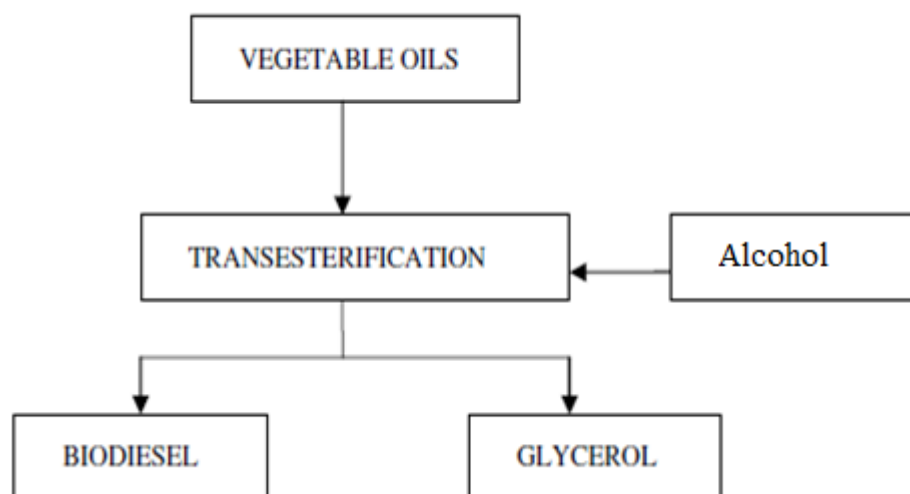


Figure 1.1. Biodiesel production sequence by transesterification.

Biodiesel is not , as such , a biotechnological creation being manufactured with any appropriate vegetable oil from harvests with no history of plant biotechnology (or even from fat of animals) by an totally chemical procedure but interpreters

include biodiesel in the portfolio of materialized bio fuels because of its biological creation as a plant seed oil. In 2005, the evaluated biodiesel world production was 2.91 million tons of oil equivalents, of which 87% was made in European Union (62% in Germany), with only the United States (7.5%) and Brazil (1.7%) as other major supplier; this total manufacture amounted to less than 20% of that of world fuel ethanol production. On the other hand, world biodiesel supply grew by threefold between 2000 and 2005 and grew from 3.2 to about 4.9 million tons from 2005 to 2006 and it is growing continuously and a marked development in the Europe as well as in United State is supposed by the International Energy Agency up to 2030.

1.2 Biodiesel feedstock

Many sources exist as the feedstock of biodiesel production, such as animal fats and vegetable oils. Vegetable oils are more widely used than other sources and are divided into two categories: (1) edible oils, such as soybean, palm, olive, sunflower, rapeseed, and corn oils and (2) inedible oils like waste cooking oil, algae, greases (float grease and trap grease), and some bean oils, such as *Jatropha curcas* (Jatropha), *Madhwa indica*, *Pongamina pinnata* (Karanja), *Hevea brasiliensis* (Rubber), and *Calophyllum inophyllum* (Polanga) (Pinzi *et al.*, 2009).

The use of edible oils in biodiesel production has been questioned because it can lead to the reduction of one of the main sources of human food worldwide. This issue will increase the price of edible oils and consequently, the price of biodiesel. The price of edible oil may increase because of the competition between the biodiesel market and human consumption. (Jain and Sharma, 2010). The raw materials price in production of biodiesel is around 60–80%.

It is predicted that environmental issues may possibly arise as the mass propagation of plants producing edible oil may lead to deforestation (Leung *et al.*, 2010). For overcoming these disadvantages, non-edible oils have attracted the researchers. Between non-edible bean oils, *Jatropha* is more beneficial and efficient for production of biodiesel in case of sociological, environmental implications and economical value (Juan *et al.*, 2010). *Jatropha curcas* is utilized for many years for hedges and medicines and also for protecting gardens and fields because the animals do not eat it (Mampane *et al.*, 1987; Gubitz *et al.*, 1999; Staubamann, *et al.*, 1999; Joubert *et al.*, 1984). The root, bark and leaves also can be used for many other pharmaceutical and industrial uses as depicted in Figure 1.3

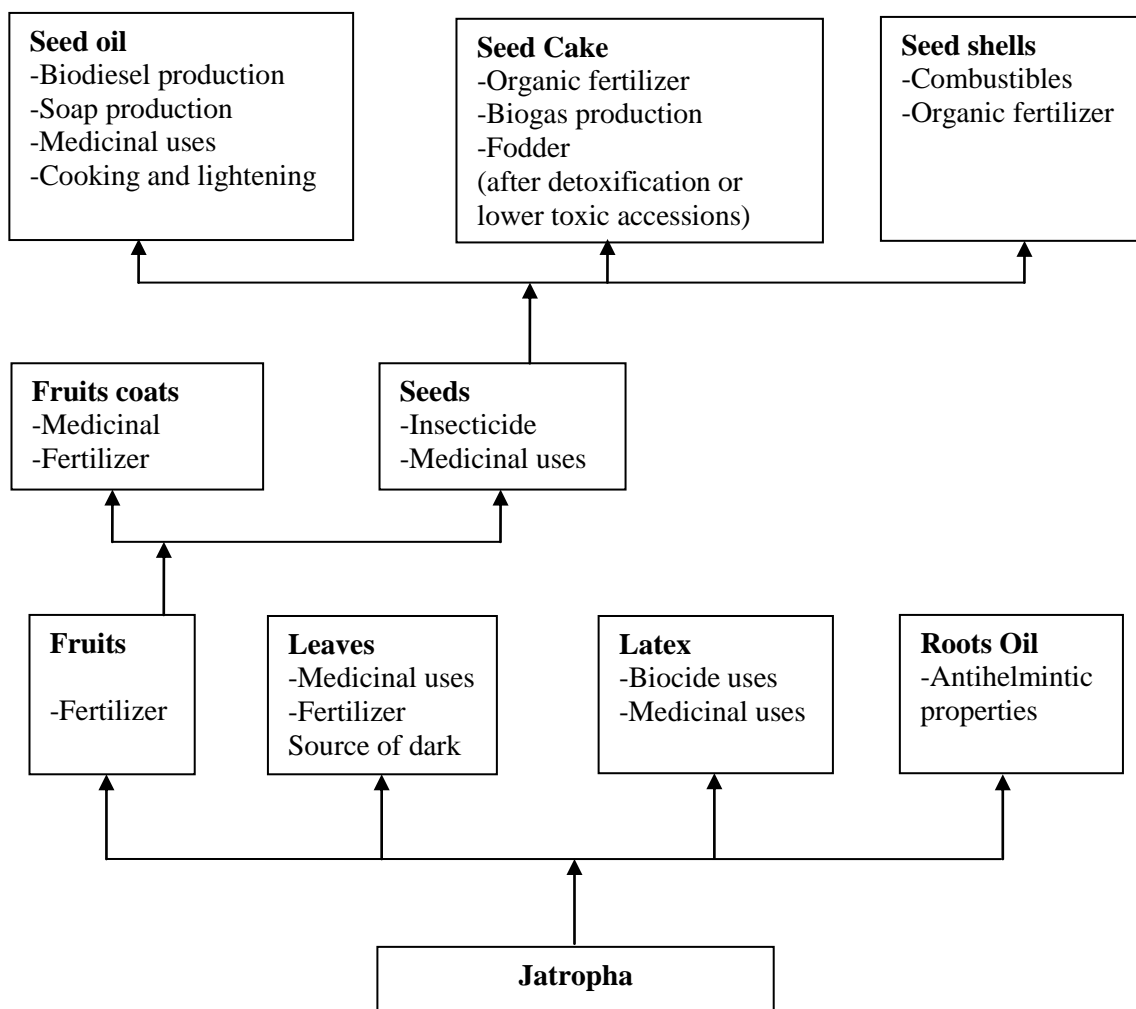


Figure 1.2. The various uses of jatropha components (adapted from Jones and Miller, 1991)

1.3 Advantages of biodiesel

In comparison with conventional diesel, biodiesel has several advantages. These advantages include: (1) CO₂ and other pollutants emission which are generated from the engines, can be reduced with help of biodiesel, (2) It is not

required to modify the engines because its properties are similar to common fuels (diesel fuel) , (3) In biodiesel with high purity, it is not needed to use lubricant, (4) Its high cetane number cause better performance in diesel engine, (5) Renewable sources are the origins of biodiesel and people can grow their own fuel, (6) It is more efficient to produce biodiesel in comparison with fossil fuels, because there will be no refinery, drilling and underwater plantation, (7) Biodiesel can be produced locally, thus it can make an area become independent of the amount of energy that is needed (Angina and Ram, 2000; Gerpen, 2005; Jain and Sharma, 2010a; Robles-Medina *et al.*, 2009 ; Jayed *et al.*, 2009).

1.4 Biodiesel production processes

It is not possible to use vegetable oils directly as a biodiesel except that mix it with diesel fuels in an acceptable ratio and this mixture of ester is stable for the using in short term. Process of mixing is not complicated and it involves blending alone and so the price of equipment is low. Due to high viscosity, using these triglyceric esters (oils) directly is impractical and unsatisfactory for using them for the long time in the engines which are working with diesel, FFA formation and acid contamination resulting in formation of gum by carbon deposition and polymerization and oxidation. Therefore oil of vegetables is processed somehow to get properties (volatility and viscosity) similar to fossil fuels properties and the fuel which is processed is possible to be used directly in the diesel engines.

To convert vegetable oil to the fuel three processing techniques are used and they are micro emulsification, transesterification and pyrolysis. Pyrolysis means change in chemical that caused by using heat to get the more uncomplicated compounds from the complex compound. This process is known as cracking. Oil of

vegetables can be cracked for improving cetane number and also decreasing in viscosity. The cracking products include carboxylic acids, alkenes and alkanes. Rapeseed, cottonseed, soyabean and other kind of oils are cracked successfully with suitable catalysts for biodiesel production (Ranganathan *et al.*, 2008). Cracking ended to good flow characteristics that achieved because of viscosity minimization. Drawbacks of this technique are high cost of equipment and also separate equipments are needed for various fractions separation. Also the product that produced was like gasoline and they contained sulfur and it cause to make the product less environmental-friendly (Ma and Hanna, 1999).

Next technique for biodiesel production is micro emulsification and it has been suggested for biodiesel production and micro emulsion biodiesel components include vegetable oil, diesel fuel, alcohol, cetane and surfactant improver in appropriate proportions (Ma and Hanna, 1999). Some alcohols like ethanol, propanol and methanol are utilized as the additives for reducing the viscosity, as the surfactants, higher alcohols are implied and as the cetane improvers alkyl nitrates are added. Reducing viscosity, good spray and cetane number increasing characters promote to use the micro emulsions but on the other hand prolong usage causes some problems such as carbon deposit formation, partial combustion and injector needle sticking (Ma and Hanna, 1999).

Another technique is transesterification which is the most common technique in biodiesel manufacturing. Biodiesel that is produced by transesterification is a liquid mixture of mono-alkyl esters of higher fatty acids. Removing component with high viscosity, glycerol, cause to reduction of product viscosity and make it similar to fossil fuels.

1.5 Reaction

1.5.1 Transesterification Process

Biodiesel fuel which is produced by alcoholysing or transesterification reaction is the substitution of ester alcohol by another alcohol in a technique much the same to hydrolysis, except that instead of water alcohol is used. Transesterification of triglycerides with alcohols is illustrated in Figure 1.4. As can be seen, at least 3 mol methanol is needed to react with 1 mol triglyceride for producing 3 mol methyl ester as the main product and 1 mol glycerol which is the byproduct (Palligarnai and Vasudevan, 2010).

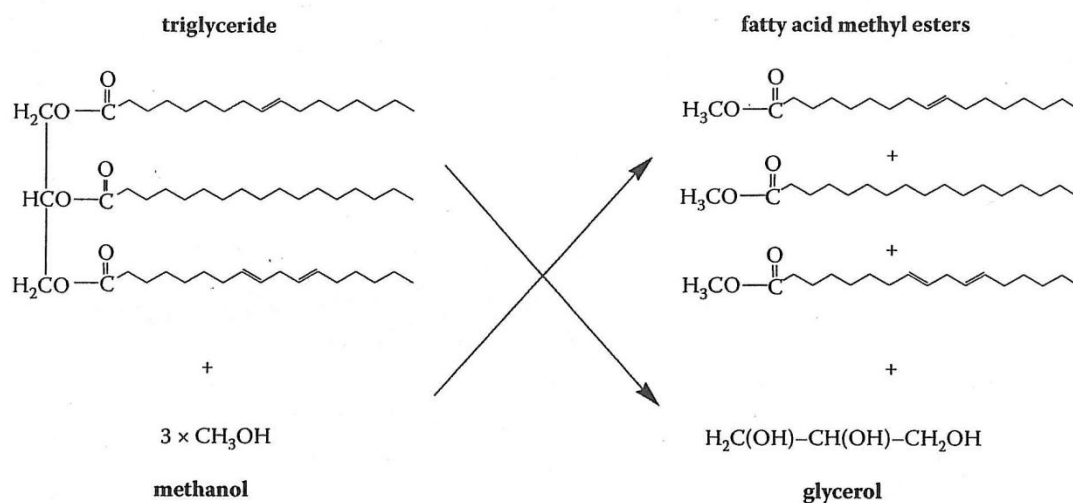


Figure 1.3. Transesterification of triglycerides with alcohols

Transesterification consists of a succession of three sequential reversible reactions. In the first step triglycerides should be converted to diglycerides, followed

by the conversion of diglycerides to monoglycerides and at the end monoglycerides to glycerol, resulting in for each glyceride, one molecule of ester at each step. The reactions are totally reversible, although the equilibrium lies in the direction of the production of glycerol and fatty acids esters. These three steps are shown in Figure 1.5 (Palligarnai and Vasudevan, 2010).

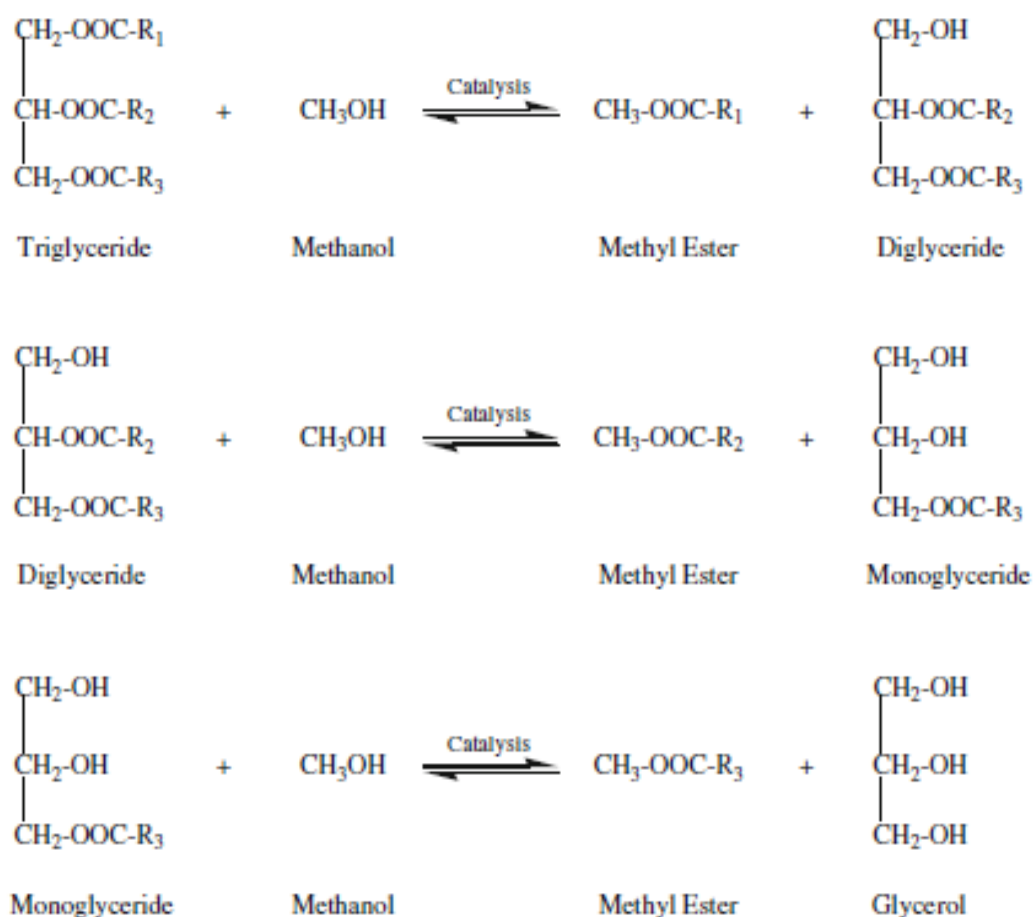


Figure 1.4. Three consecutive reversible reactions during transesterification.

The alcohols that are suitable for transesterification are methanol, butanol, propanol, amyl alcohol and ethanol. Ethanol and methanol are the most ordinary which are used, notably methanol because of its physical and chemical advantages and its low cost. This method has been widely used to decrease the viscosity of

triglycerides, thereby improving the physical properties of renewable diesel to enhance engine performance. Thus, FAMEs, known as biodiesel fuel (BDF), acquire by transesterification can be used as the alternative fuel for engines that are working by diesel.

1.6 Problem statement

An important obstacle for commercializing biodiesel is that the feedstocks for biodiesel production is substantially expensive than the cost of petroleum diesel. Production of biodiesel will become economically visible only if there was reduction in the material cost. In this case, many researchers such as Jacobson *et al.* (2007) and Chin *et al.* (2009) employed some oils such as waste cooking oils which their price is low, but still many problems exist to utilize this kind of oil, such as difficulty in oil collection. Also some times the quality of the waste cooking oil is not good enough and some pretreatment are required which this ,in turn, will increase the costs. In this research, among different sources of biodiesel, jatropha oil, an inedible oil, is chosen because of its low price and also pretreatment is not required for this kind of oil.

Jatropha oil transesterification by using alkali catalyst as well as acidic catalysts has caused many problems in biodiesel production. These are because of soap formation that easily takes place when water is produced in this reaction and also some problems such as equipment corrosion. Saponification not only lowers down the biodiesel purity also can deactivate the catalyst. Apart from that removal of these kinds of catalysts after reaction, is technically difficult and the large amount of water is needed to separate and clean the catalyst from the products. In current work, lipase catalyst is used to ignore acid and alkali catalysts disadvantages. In

addition by using biocatalyst the moderate conditions will be needed during the reaction and it helps to decreasing the process cost.

The problem with biocatalyst is inhibitor and deactivation effects of enzyme which is caused due to glycerol existence and alcohol utilization during the process. To overcome these problems and also to enhance the catalyst performance, the immobilization method is suggested.

In this study, PVA was used as the entrapment material since it has been shown to possess desirable characteristics. Due to PVA's excellent properties, PVA would serve as an excellent material to be applied in the immobilization of various microorganisms and biocatalysts. In this particular research, PVA will be used as an immobilization matrix in bead form to immobilize lipase. There are different ways of PVA-alginate preparation as immobilization matrix reported by previous studies (Dalla-Vecchia *et al.*, 2005; Imai *et al.*, 1985; Szczesna-Antczak and Galas, 2001), but such a technique cannot be used directly in this study due to the solubility of the resulting matrix in our solution.

To overcome this problem, sodium sulfate was introduced to decrease the matrix solubility after PVA-alginate beads were treated with saturated boric acid in this research. Pillay *et al.* (2005) used sodium sulfate coupled with boric acid as cross linking agent to crosslink PVA. To date, in literatures, the use of sodium sulfate has never been reported as cross-linking agent for such an immobilization matrix to immobilize *Rhizopus oryzae* lipase.

Process optimization study is important and helpful for the industrialization and development of biodiesel production (Lee *et al.*, 2011). The traditional technique of optimization studies in biodiesel manufacturing is one variable at a time, which is

based on the variation of one component at a time, with the responses serving as a function of a single parameter (Bezzera *et al.*, 2008). This method, however, is tedious and time-consuming and could not depict the total effect of the related parameters on the procedure (Bas *et al.*, 2007).

Some researchers such as Jitputti *et al.* (2006) could not find the optimum variables for maximizing biodiesel production. The reason is that they were only conducted the experiments ordinary based on the range of variables and plotted the graphs by Microsoft Excel. So the result only can show the good values and effect of variables in the reaction. Therefore, some methods such as response surface methodology (RSM), artificial neural network (ANN) and genetic algorithm (GA) could be employed in order to design and model the experimental work and also to determine the optimum condition of biodiesel production.

1.7 Research Objectives

- i. To immobilize *Rhizopus oryzae* lipase into PVA-alginate beads
- ii. To produce biodiesel from jatropha oil and study the effect of different variables (reaction time and temperature, alcohol/oil molar ratio and water content of the oil) on the yield of biodiesel
- iii. To determine optimized range of variables on biodiesel production by utilizing RSM, GA and ANN.
- iv. To determine the properties of the obtained biodiesel

1.8 Scope of research

The scopes of this research involve the following steps:

- i. Preparation and immobilization of *Rhizopus oryzae* lipase into the PVA-alginate bead
- ii. Test the catalyst activity and characterize the immobilized catalyst
- iii. Determination of the jatropha oil properties
- iv. Design of experiments based on the range of variables by design expert software
- v. Production of biodiesel through the batch process and investigate the effect of different variables on the biodiesel yield
- vi. Modeling the process and finding the optimum conditions for our process using RSM, ANN and GA
- vii. Analysis of produced biodiesel to find different physico-chemical properties of biodiesel